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Design of antibiotic containing hydrogel wound dressings: Biomedical properties and histological study of wound healing

Baljit Singh[a,*], Sushma Sharma[b], Abhishek Dhiman[a]

[a] Department of Chemistry, Himachal Pradesh University, Shimla 171005, India
[b] Department of Bio-Sciences, Himachal Pradesh University, Shimla 171005, India

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ABSTRACT

Keeping in view the antioxidant nature of the acacia gum and mucoadhesive nature of carbopol hydrogels, in the present studies, an attempt has been made to explore the potential of these materials in designing new hydrogel wound dressings meant for slow release of gentamicin, an antibiotic drug, and to enhance the wound healing potential. The hydrogel films were characterized by SEM, FTIR, XRD and swelling studies. Biomedical properties of hydrogel films like blood compatibility, antioxidant activity, mucoadhesion, antimicrobial activity, oxygen/water vapour permeability, microbial penetration and mechanical properties (tensile strength, burst strength, resilience, relaxation, and folding endurance) have been evaluated. The histological studies of wound healing were also carried out on swiss albino mice of strain Balb C and it has been observed that in case of wounds covered with hydrogel dressings shown faster wound healing, formation of well developed fibroblasts and blood capillaries as compared to open wounds. The results of biomedical properties indicated that hydrogel films are non-thrombogenic, non-haemolytic, antioxidant and mucoadhesive in nature, and are permeable to oxygen and moisture while impermeable to micro-organisms. The hydrogel wound dressings have absorbed (8.772 ± 0.184 g/g film) simulated wound fluid. Release of gentamicin drug from wound dressings occurred through Fickian diffusion mechanism in simulated wound fluid.

1. Introduction

In recent years, accumulating knowledge regarding wound healing has led to the development of numerous therapies. A plethora of novel topical preparations, dressing materials and advanced methods of debridement are now in the hands of physicians and medical experts. Wound healing is a dynamic process which normally involves systematic, coordinated and balanced activity of inflammatory, vascular, connective tissue and epithelial cells. It involves a complex series of events, lasting from the moment of injury to healing. Wounds generally produce exudate which consists of fluids, cells or other substances which slowly exuded or discharged from cells or blood vessels through small pores or breaks in cell membranes. Dry wounds tend to have higher rate of infection than moist one. Moist wound healing provides an environment that stimulates wound healing. Wound dressings are usually used to encourage the various stages of wound healing and to create better healing conditions. Wound dressings often cover the wound surface to accelerate its healing. Wound dressings have been applied to open wounds for centuries. Traditionally these were absorbents and permeable materials which could adhere to desiccated wound surface and were inducing trauma on removal. However, nowadays, new dressings have been designed to create a moist wound healing environment which allowed the wound fluids and growth factors to remain in contact with wound, thus promoting autolytic debridement and accelerating wound healing. Wound dressings are the biomaterials which promote wound healing by providing suitable micro-environment (Boateng et al., 2008). Among the wound dressings, special attentions have been given to hydrogel wound dressings due to their unique properties which can meet the essential requirements of ideal wound dressings (Higa et al., 1999).

Hydrogel dressings resemble the natural living tissue more than any other class of synthetic materials because of their high water content and soft consistency. Polysaccharide hydrogels have been observed suitable for producing flexible, mechanically strong, biocompatible, effective and economical hydrogel dressings. Hydrogel wound dressings are three-dimensional polymeric networks and are available in sheet form or as a spreadable viscous gel. Hydrogel dressings are semipermeable to gasses and water vapour. The amorphous gel formed by hydrogel dressings maintains a moist and hydrated environment (Shaheen and Yamaura, 2002; Himly et al., 1993; Saha et al., 2011). Keeping in view the antioxidant nature of the GA and mucoadhesive nature of carbopol hydrogels, in the present studies, an attempt has been made to explore

* Corresponding author. Tel.: +91 1772830944; fax: +91 1772830775.
E-mail address: baljitsinghhpu@yahoo.com (B. Singh).

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the potential of these materials in designing new hydrogel wound dressings, for slow release of antibiotic gentamicin and to enhance the wound healing potential. Biomedical properties of hydrogel films like blood compatibility, antioxidant activity, mucoadhesion, antimicrobial activity, oxygen and water vapour permeability, microbial penetration, mechanical properties (tensile strength, burst strength, resilience, relaxation, and folding endurance), and histological studies have also been evaluated.

Gum acacia (GA) polysaccharide has been extensively used in food, pharmaceutical and cosmetic industries. It is generally recognized as safe by the United States Food and Drug Administration. It has been used for the treatment of inflammation of the intestinal mucosa and to cover inflamed surfaces (Ali et al., 2009; Wapnir et al., 2008). It possesses antibacterial (Clark et al., 1993) and antioxidant activities (Al-Yahya et al., 2009; Abd-Allah et al., 2002). Topical administration of GA can inhibit lipid peroxidation in skin (Trommer and Neubert, 2005) which stimulates wound healing and angiogenesis (Altavilla et al., 2001). It is also protective against hepatic, renal and cardiac toxicities in rats (Ali et al., 2009). On the other hand, carbopol is a hydrophilic, mucoadhesive, biocompatible crosslinked polymer of polyacrylic acid (Tang et al., 2007; Renuka et al., 2012). It has been used in biomaterials as wound dressings (Renuka et al., 2012), topical (Proniuk and Blanchard, 2002) and transdermal (Arellano et al., 1999) drug delivery systems. Gentamicin sulphate is a broad spectrum antibiotic used for the treatment of infections of the skin, bones, soft tissues and wounds. It provides highly effective topical treatment in bacterial infections of the skin. It is very effective against *Streptococcus aureus* and *Pseudomonas aeruginosa* which are most commonly recovered organisms from the wounds. Gentamicin antibiotics are potent inhibitors of protein synthesis in a wide range of bacteria.

2. Materials and methods

2.1. Materials used

GA and carbopol 940 (Loba Chemie Pvt. Ltd., Mumbai, India), N,N′-methylenebisacrylamide (NN-MBA) (Acros organics, New Jersey, USA), ammonium persulphate (APS) (Qualigens Fine Chemicals, Mumbai, India), gallic acid (Himedia Laboratories Pvt. Ltd., Mumbai India), nitroblue tetrazolium chloride, riboflavin, methionine, glycerol (S.D. Fine Chemical Ltd., Mumbai, India), Folin–Ciocalteu (F–C) reagent (Merck Specialities Pvt. Ltd., Mumbai, India), bovine serum albumin (Bio Basic Inc., Canada), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, Munich, Germany), and gentamicin sulphate (Ranbaxy Lab. Ltd., New Delhi, India) were used as received.

2.2. Synthesis of hydrogel films

The solution of definite concentration of GA (5%, w/v) and carbopol (2%, w/v) was prepared and kept for 12 h for hydration. Then this solution was stirred at constant speed (100 rpm) for definite time period (45 h). Then definite concentration of NN-MBA (1.62×10^{-3} mol/L), APS (5.48×10^{-3} mol/L) and glycerol (0.14 mol/L) was added to the reaction mixture and contents were stirred for 3 h. The polymer films were prepared by solution casting method and were named as acacia-*cl*-carbopol hydrogel films. These films were washed with distilled water and ethanol to remove the soluble fraction left therein. The optimum reaction conditions were evaluated by varying the reaction parameters. The carbopol was varied from 0.5 to 2.5% (w/v), NN-MBA was varied from 1.62×10^{-3} to 11.34×10^{-3} mol/L and glycerol was varied from 0.070 to 0.47 mol/L during the synthesis of hydrogels. The optimum [carbopol], [NN-MBA] and [glycerol] were obtained 2% (w/v), 8.10×10^{-3} mol/L, and 0.34 mol/L respectively.

2.3. Characterization

SEMs were taken on FEI QUANTA 250 (Switzerland). FTIR spectra were recorded in KBr pellets on Nicolet 5700 FTIR THERMO (USA). XRD measurements were made using PAN-analytical X'Pert Pro powder diffraction system (The Netherlands). Swelling studies of hydrogels were carried out by gravimetric method (Singh and Sharma, 2009).

2.4. Drug release studies

The release profile of drug from the drug loaded polymer films was determined. The loading of a drug into the polymer matrix was carried out by swelling equilibrium method. The hydrogels were allowed to swell in solution of known concentration (1000 μg/mL) for 24 h at 37 °C and then were dried to obtain the drug loaded hydrogels. In vitro release studies of the drug were carried out by keeping the dried and drug loaded samples in definite volume of releasing medium at 37 °C temperature. The amount of drug released was measured spectrophotometrically in distilled water, PBS and simulated wound fluid after every 30 min up to 300 min in each case and then after 24 h. The absorbance of the solution of drug was measured on the UV visible spectrophotometer (Cary 100 Bio, Varian). The amount of gentamicin drug release was determined from the calibration curves prepared at λ_{max} 255 nm using UV visible spectrophotometer (Singh and Sharma, 2009). All the studies were carried out in triplicate. Based on the relative rate of diffusion of water into polymer matrix and rate of polymer chain relaxation, swelling of the polymers and the drug release profile from the drug loaded polymers have been classified into three types of diffusion mechanisms. Ritger and Peppas (1987) showed that the power law expression (Eq. (1)) could be used for the evaluation of drug release from swellable systems.

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

where M_t/M_∞ is the fractional release of drug in time t, 'k' is the constant characteristic of the drug–polymer system and 'n' is the diffusion exponent characteristic of the release mechanism. M_t and M_∞ are the amount of drug released at time 't' and at equilibrium respectively.

2.5. Biomedical properties of hydrogel wound dressings

2.5.1. Blood compatibility

The haemocompatibility was evaluated by studying the two types of blood–polymer interactions i.e. thrombogenicity and haemolytic potential. The evaluation of thrombus formation on polymeric surfaces was carried out using a gravimetric method (Imai and Nose, 1972). The haemoglobin release by haemolysis was measured by the optical density (OD) of the supernatant at 540 nm using a UV visible spectrophotometer (dos Santos et al., 2006). All studies were carried out in triplicate.

2.5.2. Antioxidant activity

Oxidative stress and excess free radical production at the wound surface impair wound healing. Different mechanisms (like free radical scavenging and metal chelation) act at different levels, independently or in combination, to bring about the wound healing effects (Akkol et al., 2011). Consequently, in the present studies, antioxidant activity of acacia-*cl*-carbopol hydrogel films was determined by F–C reagent assay, superoxide radical ($O_2^{\bullet -}$) scavenging

activity assay and DPPH radical scavenging assay methods. All studies were carried out in triplicate.

2.5.2.1. F–C reagent assay. There exists a linear correlation between the total phenolic profile and antioxidant activity (Huang et al., 2005). In this method, antioxidant activity of polymer film was measured in terms of total phenolic content. Amount of total phenolic equivalents for polymeric film was determined using F–C reagent procedure (Curcio et al., 2009). The absorbance of the solution was measured at 760 nm by UV visible spectrophotometer. The amount of total phenolic groups in each polymeric film was expressed as gallic acid equivalent and concentration of the gallic acid was measured from calibration curve.

2.5.2.2. Superoxide radical ($O_2^{\bullet -}$) scavenging activity assay. This assay method is based on the capacity of the polymeric film to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin/methionine-light system (Beauchamp and Fridovich, 1971). The polymer sample of 0.1 g was kept in distilled water for 24 h and then it was added in 1 mL reaction mixture containing 700 μL of 100 μM potassium phosphate buffer (pH 7.8), 100 μL of 130 μM riboflavin, 100 μL of 13 mM methionine, and 100 μL of 1.26 mM NBT. Reaction was started by illuminating the reaction mixture for 120 s. Reaction mixture without sample served as control, since NBT reacts with all superoxide radicals and gave maximum colour intensity. Immediately after illumination, the absorbance was measured at 590 nm. Identical test tube with reaction mixture was kept in the dark and served as blank. The percentage inhibition of superoxide anion generation was calculated using Eq. (2).

$$\%\,\text{inhibition} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100 \quad (2)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.5.2.3. DPPH radical scavenging assay. In this method, added 0.1 g of polymer and 4 mL DPPH solution (250 μM in methanol) in a test tube (as test experiment) and added 4 mL DPPH solution without polymer in another test tube (as control experiment). These test tubes were kept at room temperature (30 °C) and the absorbance was measured at λ 517 nm after every 1 h (Curcio et al., 2009). All the experiments were performed in dark. The percentage scavenging activity was calculated (Eq. (3)).

$$\%\,\text{scavenging activity} = \left[\frac{\text{control} - \text{test}}{\text{control}}\right] \times 100 \quad (3)$$

2.5.3. Mucoadhesion studies

Mucoadhesive properties of the hydrogel films were investigated by Texture Analyzer (Stable Micro Systems TA-XT2 equipped with a 5 kg load cell). The small intestine of goat was used in order to represent the mucous-like texture of a fresh wound. After thoroughly washing with saline solution, mucosal membrane was used for mucoadhesion test. The adhesive strength was determined by the maximum force (F_{max}) required to detach the film from the mucosal membrane, total work of adhesion (W_{ad}) was represented by the area under the force versus distance curve, while cohesiveness was defined as the distance travelled by film till detached. All studies were carried out in triplicate.

2.5.4. Antimicrobial activity

The antimicrobial activity of hydrogel films was determined by disk plate method against gram positive and gram negative bacteria (Rattanaruengsrikul et al., 2009). The inoculums were prepared by growing two bacterial strains of *Staphylococcus* sp. (gram positive) and *Pseudomonas* sp. (gram negative) in separate flasks in a sterilized nutrient broth medium (50 mL) and then incubated at 37 °C for 24 h. Sterilized growth media was prepared by dissolving 1.3% nutrient broth and 2.0% agar in distilled water and then this media was poured into sterilized petri plates under laminar flow and allowed to solidify. These petri plates were then incubated at 37 °C for 24 h to check any contamination. The inoculums of 12 h old culture (10 μL) of test microorganisms of *Staphylococcus* sp. and *Pseudomonas* sp. were seeded separately into respective media plates by spread plate method. Hydrogel films (unloaded and gentamicin loaded) were placed on to inoculated nutrient agar surface of the petri plates. These antimicrobial assay plates were incubated at 37 °C for 24 h. Petri plates without polymeric matrix were treated as control. The antimicrobial activity was observed by the presence or absence of zone of inhibition around the films. All studies were carried out in triplicate.

2.5.5. Oxygen permeability

The oxygen permeability of polymer films was carried out by measuring the dissolved oxygen in the distilled water as recipient using the Winkler's method (Winkler, 1888). 200 mL of distilled water was boiled for 5 min to remove dissolved oxygen and polymer films were placed on top of the flasks (test area: $4.22 \pm 0.13\ \text{cm}^2$). The negative control was the closed flask with an airtight cap preventing oxygen to enter the flask while the positive control was the open flask allowing oxygen to freely enter the flask and dissolve in the water as recipient. The test flasks were placed in an open environment under for 24 h. The results were expressed as the amount of dissolved oxygen (mg/mL) (Wittaya-areekul and Prahsarn, 2006). All studies were carried out in triplicate.

2.5.6. Water-vapour permeability

The rate of water vapour permeability of polymer films was estimated by using the method described elsewhere (Wittaya-areekul and Prahsarn, 2006). The polymeric films were placed on top of open glass vials containing 5 g of anhydrous $CaCl_2$ (test area: $1.26 \pm 0.05\ \text{cm}^2$). The positive control was air tight vial and negative control was open vial. All the vials were then placed in desiccator, containing a saturated solution of NaCl at 37 °C (approximately 70% RH). The equilibrium moisture penetration was determined by weighing the vials after specific interval of time and the rate of water vapour permeability was calculated.

2.5.7. Microbial penetration

The ability of membranes to prevent microbial penetration was tested by attaching polymeric film to the top of glass test tube (test area: $1.34 \pm 0.03\ \text{cm}^2$) containing 5 mL sterile nutrient broth (Wittaya-areekul and Prahsarn, 2006). Before test, polymeric films, nutrient broth and glass test tubes were sterilized with autoclave for 20 min at 121 °C. The negative control was a sterile nutrient broth in glass test tube closed with cotton ball while the positive control was a sterile nutrient broth in test tube open to air. The cloudiness of the nutrient broth in test tubes after 1 month of incubation at ambient environment was considered as microbial contamination. All studies were carried out in triplicate.

2.5.8. Mechanical properties

Mechanical properties, such as tensile strength, burst strength, resilience and relaxation of polymeric films were also studied using Texture Analyzer (Stable Micro Systems TA-XT2 equipped with 50 kg load cell). To evaluate tensile properties, hydrogel film of thickness 0.637 ± 0.005 mm, length 50 mm and breath 20 mm were stretched between two tensile grips until the films broke at fixed instrumental parameters. Breaking force of film (N) and extension to break of the film (mm) were determined. Percentage elongation at break, E_b, of tested films was calculated using Eq. (4), where E is the extension to break of the film (mm) and L_o is its original length

(mm). The tensile strength (T.S.) of tested films was determined using Eq. (5) (Khan et al., 2000), where F is the break force of the film and A_R (mm^2) is its cross-sectional area.

$$E_b = \frac{E}{L_o} \times 100 \quad (4)$$

$$\text{T.S.} = \frac{F}{A_R} \quad (5)$$

Burst strength test performed to evaluate maximum force required to burst the film (N) and maximum distance before bursting of film (mm) when a constant force is applied. Resilience is measurement of how a sample recovers from deformation. Resilience is the ratio of work returned by the sample as compressive strain is removed (known as recoverable work done A_2), to the work required for compression (known as hardness work done A_1) (Eq. (6)).

$$\%\,\text{resilience} = \frac{A_2}{A_1} \times 100 \quad (6)$$

Stress relaxation test was performed to evaluate viscoelastic behaviour of the material. It was evaluated by applying a constant strain for 30 s at fixed instrumental parameters. Stress relaxation was evaluated in terms of % retained force which was calculated by using Eq. (7).

$$\%\,\text{retained force} = \frac{\text{relaxed force}}{\text{force at targate distance}} \times 100 \quad (7)$$

The number of repeated folding and de-folding of a film at the same place without breaking or cracking gives the value of folding endurance (Avachat et al., 2013). All results were reported as the mean (±S.D.) of three replicates.

2.5.9. Histological studies

The wound healing characteristics of the polymer films were evaluated using a mouse model. The protocol of the present investigation was approved by Institutional Animal Ethics Committee, Himachal Pradesh University, Shimla, India. Healthy, pathogen free swiss albino mice of strain Balb C weighing 22–25 g were anesthetized with diethyl ether and the surgical area was shaved. Then a wound, approximately 1 cm^2, was created on the dorsal side of the mouse, using curved surgical scissors. Both epidermal and dermal layers were removed, creating a full-thickness wound. The animals were divided into two groups as Group I and II. The mice of the first group were wounded and wounds were kept open while in case of second group, wounds were dressed with polymer films. The animals were sacrificed at 4th, 8th and 12th day by cervical dislocation. Tissue of the wounded area was immediately excised. Haematoxylin–eosin staining procedure was employed to study the changes in wounded skin. The permanent slides were examined under Leica photoscope and were photographed.

3. Results and discussion

Optimum reaction parameters for the synthesis of hydrogel films were determined. The increase in carbopol content has not exerted very strong effect on the swelling of the polymer matrix while increase in crosslinker concentration during copolymerization reaction has decreased the swelling. This may be due to increase in crosslinking density which led to the reduction in the mobility of polymer chains. The increase in crosslinking has reduced the free volume of the hydrogel network. The amount of water uptake increased with increase in feed plasticizer content. This may be due to decrease in polymeric interactions between polymeric chains. In general, the plasticizer work by embedding themselves between the chains of polymers, spacing them apart, increasing the free volume, and thus significantly increase

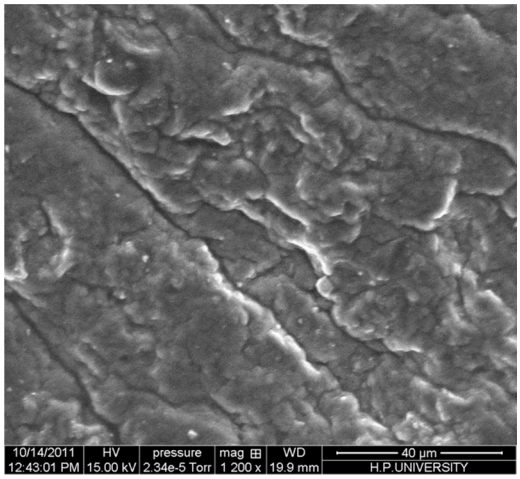

Fig. 1. SEMs of acacia-*cl*-carbopol hydrogel film in swollen state.

the swelling of the polymers (Pradhan et al., 2007). Their additions to the polymer matrix modify the mechanical and water vapour barrier properties of materials. Glycerol is an efficient plasticizer for polymer matrices. It reduces the intermolecular forces, increases the mobility of the polymer chains and also improves the absorption of exudates from wounds (Srinivasa et al., 2007). The optimum [carbopol], [NN-MBA] and [glycerol] were obtained 2% (w/v), 8.10×10^{-3} mol/L, and 0.34 mol/L respectively. The diffusion of water molecules from the polymer films occurred through Fickian diffusion mechanism. In Fickian diffusion mechanism the rate of diffusion of solvent is much less than that of the rate of polymer chains relaxation (Table 1).

3.1. Characterizations

SEMs of GA and carbopol showed smooth and homogenous morphology while in crosslinked matrix structural heterogeneity have been observed (Fig. 1). FTIR spectra of film showed the broad absorption bands between 3500 and 2900 cm^{-1} (due to —OH and —N—H stretching vibrations), at 2934.6 cm^{-1} (due to —CH_2 asymmetric stretching vibrations), at 1726.1 cm^{-1} (due to C=O stretching band which is at higher frequency than carbopol 1693.5 cm^{-1} and GA 1615.5 cm^{-1}), at 1454.4 cm^{-1} (due to C—O stretching and O—H in plane bending for acrylates), at about 1255.5 cm^{-1} (due to C—O—C asymmetric stretching for acrylates) and at 1063.0 cm^{-1} (due to asymmetric C—O—C stretching vibrations of β-(1–6) or β-(1–3)-linked galactan). These bands are observed besides the absorption peaks present in the GA and carbopol (Fig. 2). XRD patterns of all the samples have not shown any sharp peak which indicate that GA, carbopol and acacia-*cl*-carbopol polymer films are not crystalline in nature (Fig. 3) (Sahoo et al., 2011; Renard et al., 2006).

3.2. Simulated wound fluid uptake

The results of swelling showed that maximum (8.772 ± 0.184 g/g of film) simulated wound fluid uptake observed by the polymer films during the swelling. On the other hand, it is also observed that pH of the swelling medium has exerted very strong effect on the swelling of polymeric films which increased with increase in pH of the swelling medium (Fig. 4). At higher pH, ionization of —COOH groups present in the polymer matrix occurred which is responsible for ionic repulsion and expansion of polymer network and sorption of more and more wound fluid (Kawarkhe and Poddar, 2010). However, it is pertinent to mention here that the encapsulation of exudates by wound dressing is a

Table 1
Reaction parameters[a] for the synthesis of acacia-*cl*-carbopol hydrogel films and results of diffusion exponent '*n*', gel characteristic constant '*k*' and swelling in distilled water at 37 °C.

Code	Carbopol (%, w/v)	NN-MBA ($\times 10^{-3}$ mol/L)	Glycerol (mol/L)	Swelling after 24 h (g/g of gel)	Diffusion exponent[b] '*n*'	Gel characteristic constant '*k*' $\times 10^{-2}$
M_1	0.5	8.10	0.14	Not formed	–	–
M_2	1.0	8.10	0.14	6.902 ± 0.180	0.067	66.096
M_3	1.5	8.10	0.14	6.925 ± 0.143	0.228	28.525
M_4	2.0	8.10	0.14	6.979 ± 0.057	0.323	16.517
M_5	2.5	8.10	0.14	6.932 ± 0.269	0.425	8.755
M_6	2.0	1.62	0.14	8.452 ± 0.805	0.391	11.542
M_7	2.0	3.24	0.14	8.207 ± 0.605	0.371	12.720
M_8	2.0	4.86	0.14	7.973 ± 0.162	0.341	14.540
M_9	2.0	6.48	0.14	7.712 ± 0.254	0.350	13.890
M_{10}	2.0	9.72	0.14	6.308 ± 0.252	0.272	21.353
M_{11}	2.0	11.34	0.14	5.730 ± 0.267	0.275	21.042
M_{12}	2.0	8.10	0.07	6.330 ± 0.200	0.285	20.851
M_{13}	2.0	8.10	0.20	7.137 ± 0.138	0.358	14.106
M_{14}	2.0	8.10	0.27	7.236 ± 0.335	0.403	10.888
M_{15}	2.0	8.10	0.34	8.248 ± 0.197	0.450	8.337
M_{16}	2.0	8.10	0.41	8.403 ± 0.146	0.447	8.570
M_{17}	2.0	8.10	0.47	8.447 ± 0.066	0.475	7.331

[a] GA = 5% (w/v); APS = 5.48 $\times 10^{-3}$ mol/L.
[b] Fickian diffusion mechanism ($n < 0.5$).

essential property of wound dressing material by which it absorb the excess exudates from wound site and prevent the wound from mercerization. Excess exudates in wound cause growth of bacteria on wounded site which cause microbial infection and made the wound chronic. Hence, it is essential to remove the exudates from the wound site to protect the wound from mercerization (White and Cutting, 2006). In the present study, the swelling of polymer films was carried out in simulated wound fluid and indirectly this study gave the information about the ability of the films for the encapsulation of wound exudates. It is reported that moderate to high exuding wounds typically produce approximately 5 mL of exudate per 10 cm^2 in 24 h (Lamke et al., 1977). In the present case 1 g of polymer film has taken 8.772 ± 0.184 g of simulated wound fluid. The results indicate that these hydrogel wound dressings could absorb a high volume of fluid in relation to their physical dimensions and are suitable for moderate to high exuding wounds. Swelling of polymeric films in different pH buffer occurred through Fickian diffusion mechanism.

3.3. Drug release studies

The pH value within the wound-milieu influences indirectly and directly all biochemical reactions taking place in this process of healing. Hence, in the present study, an attempt has been made to study the in vitro release dynamics of the antimicrobial agent from the drug loaded polymer matrix in the different releasing medium. The release dynamics of antibiotic gentamicin sulphate from the drug loaded polymer film in different release medium is shown in Fig. 5. The release of drug closely related to the swelling characteristics of the hydrogels, which in turn, is a key function of

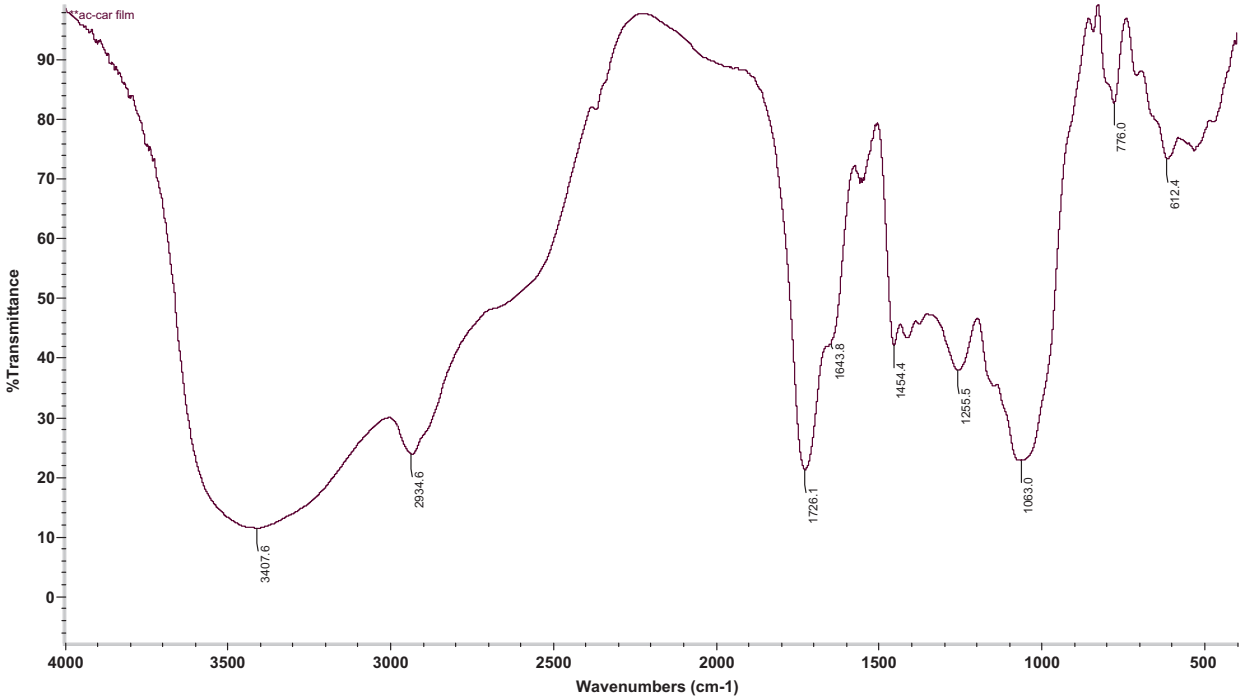

Fig. 2. FTIR spectra of acacia-*cl*-carbopol hydrogel film.

Table 2
Results of diffusion exponent '*n*' and gel characteristic constant '*k*' and various diffusion coefficients for the release of gentamicin sulphate from drug loaded acacia-*cl*-carbopol polymer films in different media at 37 °C.

Drug releasing medium	Diffusion exponent '*n*'	Gel characteristic constant '*k*' × 10^2	Diffusion coefficients (mm^2/min)		
			Initial $D_i \times 10^4$	Average $D_A \times 10^4$	Late time $D_L \times 10^4$
Distilled water	0.342	13.011	2.273	10.075	4.163
Phosphate buffer saline	0.509	5.416	4.609	6.481	6.287
Simulated wound fluid	0.483	4.697	2.623	3.095	2.944

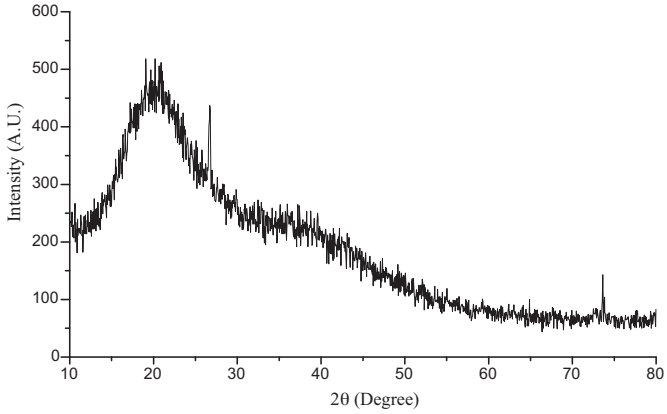

Fig. 3. X-ray diffraction patterns of acacia-*cl*-carbopol hydrogel film.

chemical architecture of the hydrogels. The diffusion of drug from polymeric matrix occurred through Fickian diffusion mechanism in all mediums (Table 2). Fickian diffusion occurs when the rate of diffusion is much less than that of relaxation. When the drug is loaded into the hydrogels by equilibrium swelling in the drug solution, drug release from the swollen gel follows Fick's law. The values of average diffusion coefficients have been observed higher than the late diffusion coefficients. These values show that in the earlier stages the rate of diffusion of drug from the polymer was higher than the later stages. This may be due to higher concentration gradient. It means after maintaining the certain concentration, the release of drug from the polymer matrix occurred slowly and this is very important observation for designing the controlled drug delivery systems. These results indicate that hydrogel with drug can significantly improve wound healing as compared to the hydrogel without drug (Hwang et al., 2010).

3.4. Biomedical properties of hydrogel wound dressings

3.4.1. Blood compatibility

These films were designed to be used topically in wound healing. It is important to evaluate their tissue and blood compatibility. Furthermore, the thrombogenic character is a desirable property required in materials to be used as wound dressing. Once the protein adhesion constitutes the first step to initiate the coagulation cascade, material can accelerate thrombus formation, stop haemorrhage and helping in the healing process. In the present study the clot formed in the positive control was weighed 0.275 ± 0.010 g and in test experiment of the films was (0.234 ± 0.018) g (Table 3). The results showed that when blood was kept in contact with polymeric film, slightly less amount of clot was formed indicating this polymeric film as non-thrombogenic (Imai and Nose, 1972). Similar results have been reported for chitosan and gelatine based wound dressings (dos Santos et al., 2006; Dawlee et al., 2005). The results of haemolysis showed that the polymer films haemolytic index is 3.27 ± 0.49% and it is classified as non-haemolytic in nature. The extent of haemolysis being lower than the permissible level 5%, (Dawlee et al., 2005) and hence, these polymeric films can act as suitable candidate for wound dressing.

3.4.2. Antioxidant activity

Antioxidants are organic substances that are capable of counteracting the damaging effect of oxidation in animal tissues. These substances significantly decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans. Free radicals are produced

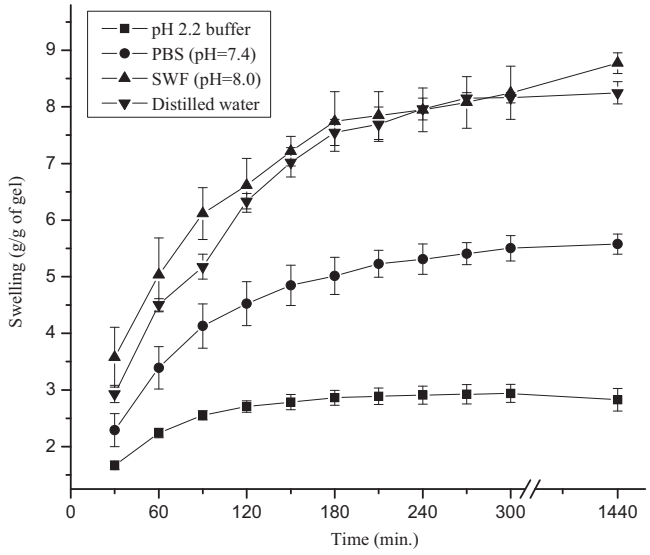

Fig. 4. Effect of pH of swelling medium on swelling of acacia-*cl*-carbopol hydrogel films at 37 °C.

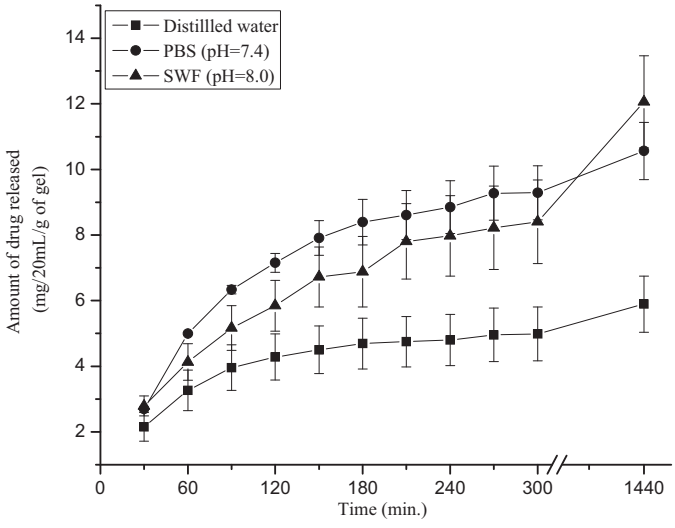

Fig. 5. Release profile of gentamicin sulphate from drug loaded acacia-*cl*-carbopol polymer films in different medium at 37 °C.

Table 3
Results of biomedical properties of acacia-*cl*-carbopol hydrogel films.

Thrombogenicity	Weight of blood clot (g)	Thrombose (%)	Inference
	0.234 ± 0.018	84.97 ± 6.39	Non-thrombogenic
Haemolysis	OD at λ_{max} = 540 nm	Haemolytic index (%)	Inference
	0.7888 ± 0.0212	3.27 ± 0.49	Non-haemolytic
F–C reagent assay	Weight of film (g)	Gallic acid equivalent (μg)	Inference
	0.1	18.63 ± 0.49	Antioxidant
$O_2^{\bullet -}$ radical scavenging assay	Weight of film (g)	Inhibition (%)	Inference
	0.1	65.32 ± 0.34	Antioxidant
DPPH scavenging assay	Weight of film (g)	Scavenging (%)	Inference
	0.1	89.64	Antioxidant
Mucoadhesion[a]	Maximum detachment force F_{max} (mN)	Work of adhesion, W_{ad} (mN mm)	Detachment distance (mm)
	93.00 ± 5.29	29.33 ± 7.37	3.25 ± 0.48
Tensile strength[b]	Breaking force (N)	Tensile strength (N (mm)$^{-2}$)	Elongation at break (%)
	9.251 ± 1.781	0.726 ± 0.134	235 ± 22.71
Burst strength[c]	Dimension of film (cm^2)	Bursting strength (N)	Distance at burst (mm)
	3 × 3	12.65 ± 1.06	11.99 ± 1.43
Resilience[d]	Dimension of film (cm^2)	Resilience (%)	Inference
	3 × 3	17.66 ± 2.85	Less resilient
Relaxation[e]	Dimension of film (cm^2)	Force at target distance (N)	% retained force
	3 × 3	0.40 ± 0.06	55.49 ± 9.22
Oxygen permeability	Thickness of polymeric film (mm)	Oxygen present in flask covered with film (mg/L)	Inference
	0.60 ± 0.03	5.63 ± 0.06	Permeable
Water vapour permeability	Thickness of polymeric film (mm)	Rate of water vapour penetration (mg/day/L)	Inference
	0.64 ± 0.03	1252.88 ± 320.73	Permeable
Microbial penetration	Time (days)	Positive control (turbidity)	Polymeric films and negative control (turbidity)
	1	No	No
	2	Light	No
	14	Clear	No
	30	Complete	No

[a] Contact time = 60 s, return distance = 15.0 mm, applied force = 0.10 N, trigger force = 0.029 N, test speed = 0.10 mm/s, pre test speed = 0.50 mm/s, post test speed = 0.10 mm/s.
[b] Trigger force = 0.029 N, test speed = 2.00 mm/s, pre test speed = 0.20 mm/s, post test speed = 10 mm/s.
[c] Distance = 15.0 mm, trigger force = 0.049 N, test speed = 1.0 mm/s, pre test speed = 2.0 mm/s, post test speed = 10 mm/s.
[d] Distance = 2.0 mm, trigger force = 0.049 N, test speed = 0.5 mm/s, pre test speed = 1.0 mm/s, post test speed = 0.5 mm/s.
[e] Distance = 2.0 mm, trigger force = 0.049 N, test speed = 0.5 mm/s, pre test speed = 1.0 mm/s, post test speed = 10 mm/s.

by leukocytes to kill invading bacteria in wounds. They are generated during inflammation reactions. When overproduced, free radicals activity results in impairment of cellular functions as in wound healing (Opoku et al., 2007). Therefore, if a material used in wound dressing has an antioxidant property then it will aid in wound healing due to scavenging of overproduced free radicals and thus stop the impairment of cellular functions due to free radicals.

Results of F–C reagent assay method for antioxidant activity showed that acacia-*cl*-carbopol films show antioxidant activity. It has been observed that 0.1 of polymeric film shown gallic acid equivalent concentration 18.63 ± 0.49 μg. The results of superoxide radical ($O_2^{\bullet -}$) scavenging activity assay showed that for 0.1 g of polymeric film 65.32 ± 0.34% inhibition of superoxide radicals occurred during the test. The results of DPPH radical scavenging assay method showed the decrease in absorbance of DPPH radical and 89% scavenging of DPPH radicals after 9 h by 0.1 g of polymer film which indicated the antioxidant nature of the polymer matrix (Table 3). All these results suggested that polymeric film has antioxidant activity and it will enhance the wound healing effect of wound dressing (Moseley et al., 2003). The antioxidant nature of the polymer films may be due to the presence of GA in the hydrogel wound dressings. GA is a potent superoxide scavenger and exerts the protective effect against cardiotoxicity induced by doxorubicin in mice and urinary bladder tissue damage resulting from cyclophosphamide treatment in a rat. Scavenging of the reactive oxygen species (ROS) and NO to limit the oxidative damage to the cell membrane appears to be the first line of action for GA (Al-Yahya et al., 2009; Abd-Allah et al., 2002).

3.4.3. Mucoadhesion studies

Adhesion of wound dressing on wounded skin is of a great importance for the efficiency of topical wound treatment. During the mucoadhesion studies, three parameters were measured: (i) work of adhesion, W_{ad} (work required to overcome the attractive forces); (ii) stickiness factor (maximum detachment force F_{max} required for detachment of hydrogel from mucosal surface); and (iii) cohesiveness (the distance hydrogel travels before detachment). All of these factors are correlated with the strength of the bonds formed between the polymeric matrix and mucosal membrane during the contact time (Kianfar et al., 2013). F_{max} and W_{ad} for polymeric film were observed 93.00 ± 5.29 mN and 29.33 ± 7.37 mN mm respectively. When probe was moved upward after applying force, hydrogel film did not detach upto 3.25 ± 0.48 mm (Table 3). Salcedo and coworkers have observed 2.75 ± 0.10 mN mucoadhesive potential for chitosan (Salcedo et al., 2012).

3.4.4. Antimicrobial activity

Presence of inhibition zones against both bacterial strains was observed in case of gentamicin loaded polymeric films. Larger zone of inhibition occurred against gram negative bacteria than gram positive. This may be due to more activity of gentamicin against gram negative bacteria. However, unloaded polymeric films have shown inhibition zone only for gram positive bacteria indicating antimicrobial nature of the hydrogel wound dressings. These results suggest that these hydrogel films may reduce the chance of secondary bacterial infection in wounds effectively.

3.4.5. Oxygen permeability

The tested solutions from airtight flask (negative control) and opened flask (positive control) had dissolved oxygen 4.0 mg/L and 7.1 mg/L, respectively, whereas those flasks covered with acacia-*cl*-carbopol polymer films had dissolved oxygen 5.63 ± 0.06 mg/L, respectively. The negative control group had significantly lower dissolved oxygen values than test group, but dissolved oxygen value did not reached high values as the open control (Table 3). Similar, observation has been made in case of sericin/collagen based wound dressings (Akturk et al., 2011). These results indicate that polymer films are permeable for oxygen and these films could provide adequate supply of O_2 to the wound bed which will help in proper maintenance of wound. Low oxygen concentration decreases the regeneration of tissue cell or makes possible the proliferation of anaerobic bacteria. Adequate supply of oxygen to the wound tissue is vital for optimal healing and resistance to infection (Gottrup, 2004).

3.4.6. Water-vapour permeability

The results of the water vapour permeability of open vial and vial closed with polymer films were found to be 11,034.80 and 1252.88 ± 320.73 mg/day/L respectively. Water vapour permeability of polymer films is significantly less than open vial (Table 3). So it can efficiently prevent excessive dehydration from wound surface and maintain moist environment. According to winter's studies those dressings which retain moist wound environment have many advantages over those dressings which create dry wound environment. Moist wound environment prevents tissue dehydration, which helps in earlier epithelialisation, accelerates angiogenesis, increases breakdown of dead tissue and fibrin contributing to autolytic debridement, potentates interaction of growth factors with their target cells, reduces the incidence of infection and less painful in nature (Winter, 1963). Use of more moisture-retentive dressings generally achieves environments supportive of earlier healing outcomes when compared to less moisture-retentive dressings. Evidence further suggests that greater dressing

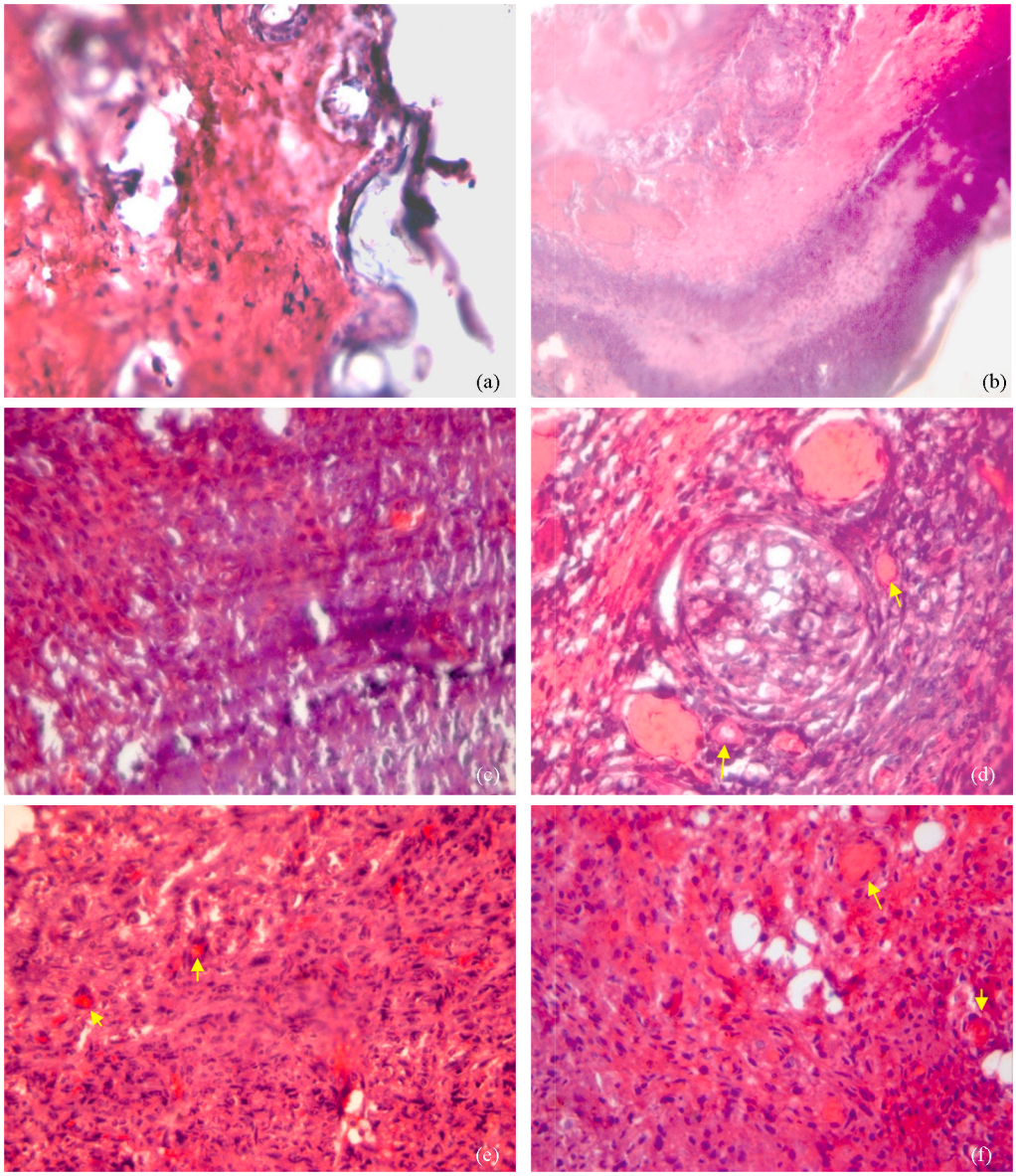

Fig. 6. (a) Skin tissue after 4th day of wound in case of open wound and (b) inflammation of skin tissue after application of hydrogel film after 4th day of wound. (c) Open wound exhibiting few capillaries in comparison to (d) treated tissue with well developed blood vessels at day 8. (e) Little collagenous material and blood cells as compared to the (f) treated tissue at day 12.

moisture retention is associated with fewer clinical infections, greater patient comfort, and reduced scarring (Bolton et al., 2000).

3.4.7. Microbial penetration

Wounds provide a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. The influence of infection on wound healing has been well reported (Greenhalgh et al., 1986). Infections from the external environment retard the rate of wound healing significantly. So, wound dressings should protect the wound from penetration of pathogenic microbes from the external environment to wound surface. The results showed that only the positive control test tubes had bacterial contamination, whereas, neither negative control nor the acacia-*cl*-carbopol polymer films have shown visible microbial penetration during one month period (Table 3). Microbial impermeability of films will reduce the chance of secondary bacterial infection in the wounds making it more suitable for wound dressing application as compared to the conventional wound dressings such as gauze dressings. It is also reported in literature that 64 layers of gauze were not sufficient to prevent the entry of exogenous bacteria into the wound and moistened gauze is even less effective barrier to bacteria (Baranoski and Ayello, 2012).

3.4.8. Mechanical properties

The tensile test provides an indication of the strength and elasticity of the film, which can be reflected by tensile strength and elongation at break. It is suggested that films which are suitable for wound dressings should be strong and flexible (Devi et al., 2012). Results showed that these hydrogel films were highly flexible in nature and have elongation at break $235 \pm 22.71\%$ and tensile strength $0.726 \pm 0.134\,N\,mm^{-2}$. These films were found to have much higher elongation break than some reported wound dressings (Zaman et al., 2011). Flexibility is required to overcome strains and stresses on body and only a flexible dressing could sustain various mechanical stresses without breaking. Burst strength for polymer films is presented in Table 3. Bursting strength 12.65 ± 1.06 N and distance at burst 11.99 ± 1.43 mm was obtained for polymeric films.

Resilience is how well a product fights to regain its original position. The film showed $17.66 \pm 2.85\%$ resilience. Stress relaxation is the loss in stress when it is held at a constant strain over a period of time. It is usually expressed in terms of percent stress remaining after an arbitrary length of time at a given temperature. It is an important property where a given level of force or tension must be maintained over a long time, like if elbow is folded. Retained force for hydrogel film was observed $55.49 \pm 9.22\%$. Folding endurance values for films was observed more than 300, which indicates high mechanical strength of these films (Table 3). This is highly desirable because it would not allow easy dislocation of the films from the site of application or breaking of films during administration (Avachat et al., 2013).

3.4.9. Histological studies

The results of histological slides studies showed that wound healing occurred at faster rate in case of wounds covered with polymer films as compared to the open wounds. The complete wound healing occurred after 12 days in case of mice where the wound dressings were used. It was also observed that during wound healing in mice, the hydrogel films were easily removed from the wound surface and dressings did not adhere to the wound bed and film removal didn't results into the loss of tissue at the wound site. The histological observations of the wound tissue in the three groups are shown in Fig. 6. With polymer matrix, inflammatory response was more pronounced on postoperative day 4 and then it was gradually subsided (Gál et al., 2009). On day 8, blood vessels and capillaries appeared in dressed wound but no such capillaries were visible in open wound. After 12th days, fibroblasts were well developed, thickened collagen fibres (Noorjahan and Sastry, 2004) and blood capillaries (Murakami et al., 2010; Al-Henhena et al., 2011) were formed, while in the open wound, no such changes were observed. Thus well developed connective tissues were visualized in the hydrogel treated wounds. All these observations indicated that polymer wound dressings have exerted positive effects on wound healing processes in mice skin tissue.

4. Conclusion

The blood compatibility and antioxidant activity of the polymeric films are contributing factors for improved wound healing in the treated mice. The results of simulated wound fluid uptake and release of antibiotic in wound fluid indicate that these hydrogel wound dressings could absorb high volume of fluid and are suitable for moderate to high exuding wounds and could release the antibiotic drugs in controlled manner. This will further enhance the wound healing potential of the hydrogel wound dressings. Permeability to gaseous and moisture of the dressings can maintain the wound environment besides protecting it from external injury. All these results indicate that acacia-*cl*-carbopol polymeric films may be used as wound dressings for the slow release of antibiotic drug to the wounds.

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